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Dehydrogenation: A Previously Unreported Pathway of Lindane Metabolism in Mammals

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Environmental Protection Agency

ROBERT W. CHADWICK, LUCY T. CHUANG, AND KATHERINE WILLIAMS

Environmental Protection Agency, National Environmental Research Center, Pesticides and Toxic Substances Effects Laboratory, Research Triangle Park, North Carolina 27711

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This study presents evidence for the dehydrogenation of lindane by a hepatic microsomal mixed-function oxidase system. Preliminary investigation established that the incubation of lindane with rat liver homogenates produces a chlorinated, nonpolar compound identified as hexachlorocyclohexene. Differential centrifugation resulted in the sedimentation of most of the dehydrogenase activity in the microsomal fraction. Optimum *in vitro* assay conditions were established and it was found that the dehydrogenase system required molecular oxygen and reduced pyridine nucleotide coenzyme for maximum activity. Inhibition by SKF 525-A and CO suggested that the enzyme was cytochrome P-450 dependent. Lack of inhibition by cyanide indicated that the cytochrome b_5 desaturase system was probably not involved. Pretreatment of rats with DDT, which stimulates lindane metabolism, also induced significantly higher dehydrogenase activity. Both the *in vivo* and *in vitro* metabolism of hexachlorocyclohexene produced previously identified lindane metabolites. The existence of a cytochrome P-450 dependent mixed-function oxidase which catalyzes the dehydrogenation of lindane has not previously been reported and may be of importance in the metabolism of other xenobiotics.

It is generally accepted that dehydrochlorination of lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane, γ-HCH[1]) to γ-2,3,4,5,6-pentachlorocyclohex-1-ene (γ-PCCH) is the first step in the metabolism of this organochlorine insecticide by both plants (1, 2) and animals (3–14). In 1965 Grover and Sims showed that rats metabolize both lindane and γ-PCCH to 2,3,5 and 2,4,5-trichlorophenol which are excreted in the urine as free phenols, sulfates, and glucuronic acid conjugates (14). Because the metabolites identified in their study resembled those from the degradation of 1,2,4-trichlorobenzene in rabbits (15), the authors speculated that lindane metabolism proceeds via γ-PCCH as an intermediate step to 1,2,4-trichlorobenzene. Currently, most of the published schemes of lindane metabolism are based on this work by Grover and Sims (16–18) and indicate that lindane is metabolized almost exclusively by dehydrochlorination through the intermediate metabolite γ-PCCH. Nevertheless, recent identification of some previously unreported lindane metabolites in flies (11), and the urine of both rats (19) and rabbits (20) led to speculation that there might be important metabolic path-

[1] The following abbreviations are used: γ-HCH, γ-1,2,3,4,5,6-hexachlorocyclohexane; γ-PCCH, γ-2,-3,4,5,6-pentachlorocyclohex-1-ene; HCCH, Hexachlorocyclohexene; PCCOL, 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol; G6-P, Glucose-6-phosphate; GSH, glutathione; DEGS, diethyleneglycosuccinate.

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ways other than the accepted dehydrochlorination of lindane through γ-PCCH. This study presents evidence to support the existence of a different initial metabolic pathway, specifically the dehydrogenation of lindane to the intermediate metabolite, hexachlorocyclohexene (HCCH).

MATERIAL AND METHODS

Animals

Weanling female Sherman and Sprague–Dawley rats were used in this study. The animals were housed in individual metabolism cages and when treated received po injections of peanut oil solutions of the compounds investigated. Since the rate of lindane metabolism *in vitro* is very low in livers from normal rats, optimum assay conditions were established using liver preparations from rats treated po with 2 mg DDT/day for 2 wk. All animals were sacrificed by decapitation to avoid any complications due to anesthetics.

Chemicals

All pesticides and pesticide metabolites except 1,2-dichlorochlordene, which was provided by Velsicol Chemical Corporation, Chicago, Ill. and those synthesized or isolated from rat urine, were obtained from the Pesticides and Toxic Substances Effects Laboratory, Analytical Reference Standards Repository, Research Triangle Park, N. C. Hexachlorocyclohexene (HCCH) was synthesized by photochlorination of *o*-dichlorobenzene (21). It was isolated by elution with hexane from a silicic acid column. Comparison of synthetic HCCH with the metabolite extracted from liver incubation mixtures, employing lindane as the substrate, demonstrated that the compounds are identical. The mass spectra of the compounds have the same molecular ion, chlorine isotope distribution, and fragmentation pattern. While complete characterization of this intermediate metabolite will be reported in another publication, the salient ions and their intensities include ($M^{\dagger}$286 m/e (3%), $M^{\dagger} - Cl$ 251 m/e (6%), $M^{\dagger} - [Cl + HCl]$ 251 m/e (83%), $M^{\dagger} - C_3H_2Cl_3$ 190 m/e (2%), $M^{\dagger} - [Cl_2 + HCl]$ 180 m/e (74%)). The compounds have identical retention times on the different glc packing materials used in this study and both elute similarly from a silicic acid column. The lindane metabolite 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol (PCCOL) was isolated from urine and identified previously (19). Dithiothreitol was obtained from Calbiochem, San Diego, Cal. Nicotinamide, glucose-6-phosphate (G6-P), glutathione (GSH), cysteine, G6-P dehydrogenase, NAD^+, NADH, $NADP^+$, NADPH, and Trizma preset pH crystals for Tris-HCl buffer were purchased from Sigma Chemical Company, St. Louis, Mo. SKF 525-A was purchased from Smith, Kline, and French Laboratories, Philadelphia, Pa. EDTA was obtained from Fisher Scientific Company, Pittsburgh, Pa. 1.5% OV-17 + 1.95% QF-1, on 80/100 mesh Gas Chrom Q was obtained from Applied Science Laboratories Inc., State College, Pa. Diethyleneglycosuccinate (DEGS) and Gas Chrom Q were obtained from Supelco, Inc., Bellefonte, Pa. Gas chromatographic grade solvents were used for all extractions.

Tissue Preparation

Livers were quickly removed, weighed, and a 1 g portion was homogenized with 5 ml of 0.1 *M* Tris-HCl buffer, pH 7.5, in a Potter–Elvehjem homogenizer with a motor driven Teflon pestle. The homogenization was carried out at 4°C for 1 min. The homogenate was centrifuged at 9000*g* for 20 min in a refrigerated Sorval superspeed centrifuge. The reddish supernatant was used for most of the assays conducted in this study. When microsomes were isolated, the 9000*g* supernatant was centrifuged at 105,000*g* for 75 min in a refrigerated Beckman Model L-2 Ultracentrifuge. The resulting glassy-red pellet was well drained

and homogenised by hand into a volume of 0.1 *M* Tris-HCl buffer equal to the volume of the solution that was centrifuged at 105,000*g*. Protein concentration was determined by the Lowry method (22).

Enzyme Assays

The standard incubation mixture of 1 ml of the 9000*g* supernatant (or resuspended microsomes) plus 2 ml of an aqueous mixture containing 160 μmol of Tris-HCl buffer, pH 7.5, 60 μmol of nicotinamide, 2 μmol of $NADP^+$, 10 μmol of G6-P, 0.4 units of G6-P dehydrogenase, and 0.34 μmoles of lindane dissolved in 0.1 ml of acetone. The mixture, in a 10 ml Erlenmeyer flask, was incubated for 30 min in a Dubnoff Metabolic Shaking Incubator at 150 oscillations/min and 37°C. The reaction was terminated by the addition of 1.5 ml of 1 *N* HCl to the incubation mixture. In other experiments, acetone solutions of γ-HCCH, cis- and trans-chlordane, and α-, β- and δ-hexachlorocyclohexane were incubated as substrates in place of lindane. In the determination of V_{max} and K_m values, a 20 min incubation time was used.

Extractions

The incubation mixture was transferred to a 15 × 125 mm screwcapped test tube containing approximately 1 cm of NaCl in the bottom of the tube. The incubation flask was rinsed with 1.5 ml of 1 *N* HCl followed by 5 ml of benzene. Both the HCl and the benzene were transferred to the test tube containing the incubation mixture. The combined mixture in the test tube was then shaken vigorously on a vortex mixer for 1 min. The resulting emulsion was broken by centrifugation at 3000 rpm for 3–5 min and the benzene was pipetted off. The extraction was repeated once more and the combined extracts were analyzed by gas chromatography.

Chromatography

Metabolites were assayed on a Micro-Tek MT-220 gas chromatograph equipped with an electron-capture detector and a Coulson electrolytic conductivity detector. The columns used for analysis were 6 ft × 0.25 in. glass U-tubes packed with either 1.5% OV-17 + 1.95% QF-1, on 80/100 mesh Gas Chrom Q or 5% DEGS on 80/100 mesh Gas Chrom Q and both were isothermally maintained at 170°C. The flow of nitrogen carrier gas was maintained at 50 cm^3/min.

Statistics

Data were analyzed by the use of Student's *t* test.

RESULTS

In Vitro Metabolism of Lindane in the Rat

Preliminary investigation established that the incubation of lindane with rat liver homogenates produced a chlorinated, nonpolar metabolite which had an identical mass spectra and identical GLC retention time to synthetically prepared hexachlorocyclohexene. The metabolite also eluted from silicic acid similarly to hexachlorocyclohexene. Consequently, the *in vitro* dehydrogenase activities of both control and induced animals were investigated and compared.

Intracellular Distribution

Differential centrifugation resulted in the sedimentation of the highest specific dehydrogenase activity in the microsomal (105,000*g*) pellet as indicated in Table 1.

Optimum Assay Conditions

The 9000*g* supernatant from the centrifugation of rat liver homogenates was used to establish optimum *in vitro* assay conditions for the dehydrogenase system.

Effect of time. Figure 1 indicates that the amount of HCCH formed during the dehydrogenation of lindane was reasonably linear up to 30 min and leveled off thereafter.

TABLE 1

Microsomal Dehydrogenase in Subcellular Fractions Prepared by Differential Centrifugation from Homogenates of Rat Liver

Fraction[a]	Total protein (mg)	Total activity (μg HCCH/ 30 min)	Relative sp act[b]
Homogenate	28	0.299	0.16
Mitochondrial pellet	24	0.069	0.04
9000*g* Supernatant	23	0.496	0.32
Microsomal pellet	6	0.430	1.00
105,000*g* Supernatant	17	0.022	0.01

[a] Preparation of subcellular fractions and incubation conditions as described in Methods.

[b] Relative sp act = (Total activity of fraction/ fraction total protein)/(Total activity of Microsomal Pellet/Microsomal Pellet Protein).

Effect of pH. The optimum pH for the dehydrogenase system was 7.5 as indicated in Fig. 2.

Effect of protein concentration. As illustrated in Fig. 3, dehydrogenase activity was proportional to microsomal protein concentration at the level routinely employed in the assay (0.2*g* wet liver wt or 23 mg of protein in the aliquot of the 9000*g* supernatant taken for analysis).

Effect of substrate concentration. The apparent Michaelis constants (K_m) for the dehydrogenase systems from both control

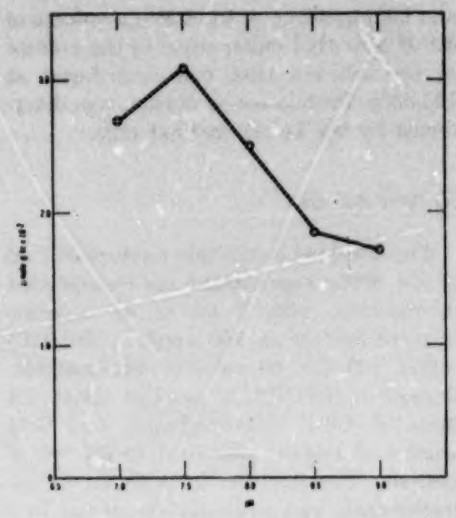

FIG. 2. *Effect of pH on the* in vitro *metabolism of lindane to hexachlorocyclohexene (μmole/gram liver/ hour × 10^{-3}). Incubation conditions as described in Methods. Every value represents the mean of two determinations on each of the liver preparations from two rats.*

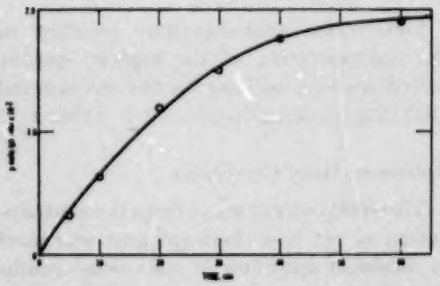

FIG. 1. *Effect of time (minutes) on the* in vitro *metabolism of lindane to hexachlorocyclohexene (μmole/gram liver × 10^{-3}). Incubation conditions as described in Methods. Each value is the mean of two determinations.*

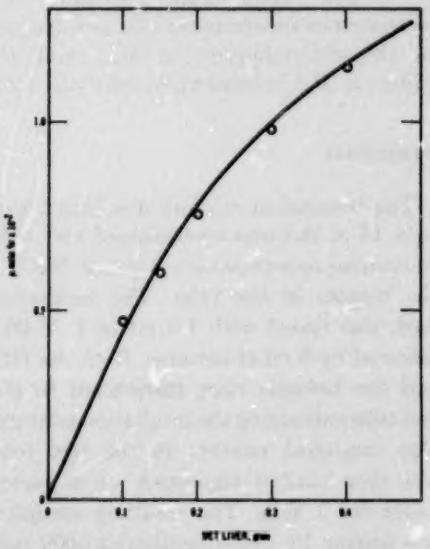

FIG. 3. *Effect of protein concentration (grams wet liver) on the* in vitro *metabolism of lindane to hexachlorocyclohexene (μmole/hour × 10^{-3}). Incubation conditions as described in Methods. Each value is the mean of two determinations.*

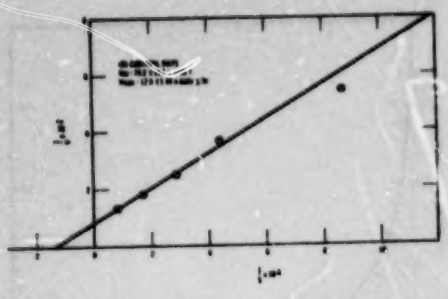

FIG. 4. *Lineweaver–Burk plots for the* in vitro *metabolism of lindane to hexachlorocyclohexene by control rats. S = substrate added (mole/liter); V = product formed (μmole/gram/hour). Incubation conditions as described in Methods. Every value is the mean of two determinations on each of the liver preparations from three rats.*

(Fig. 4) and DDT-treated rats (Fig. 5) were determined from the double reciprocal plots of Lineweaver and Burk (23). The apparent mean $K_m \pm$ SE for the DDT and control animals, 20.0 ± 0.60 and 79.2 ± 10.5 μmol of lindane per liter, respectively, were significantly different at $P < 0.01$. The lower K_m value of the DDT treated rats could be the result of a higher affinity between the substrate lindane and the enzyme system, the induction of a new enzyme or perhaps a "deinhibition" of the dehydrogenase enzyme system in these animals. The maximal velocity ($V_{max} \pm$ SE) of the control animals (12.5 ± 1.4 nmol HCCH formed/g/h) was increased more than threefold (41.2 ± 10.7 nmol HCCH formed/g/h) by the DDT treatment.

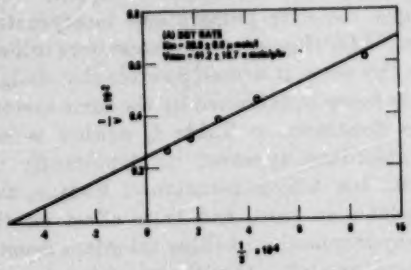

FIG. 5. *Lineweaver–Burk plots for the* in vitro *metabolism of lindane to hexachlorocyclohexene by DDT treated rats. S = substrate added (mole/liter); V = product formed (μmole/gram/hour). Incubation conditions as described in Methods. Every value is the mean of two determinations on each of the liver preparations from five rats.*

TABLE 2

The Coenzyme Requirements of the Dehydrogenase System from Rat Liver

Incubation medium[a]	Relative dehydrogenase activity
Complete 9000*g* supernatant	100
– dehydrogenase enzymes	0
– dehydrogenase enzymes plus enzymes heated at 100°C, 2 min	0
– Nicotinamide (60 μmol)	82
– Nicotinamide (60 μmol) plus NADPH generating system	6
– NADPH generating system	44
– NADPH generating system plus NADH (2 μmol)	90
– NADPH generating system plus NADPH (2 μmol)	90
– NADPH generating system plus NAD (2 μmol)	78
– NADPH generating system plus NADP (2 μmol)	67
Complete microsomal preparation	100
– NADPH generating system plus NADH (2 μmol)	29
– NADPH generating system plus NADH (10 μmol)	40
– NADPH generating system plus NADH (20 μmol)	48
– NADPH generating system plus NADPH (2 μmol)	48
– NADPH generating system plus NADPH (10 μmol)	115
– NADPH generating system plus NADPH (20 μmol)	118

[a] "Complete" incubation medium and conditions as described in Methods.

Requirements of the dehydrogenase system. As shown in Table 2, the dehydrogenase system required reduced pyridine nucleotide for complete activity and NADPH appeared to be preferred over NADH. While both NADH and NADPH provided almost maximal activity with the 9000*g* supernatant, NADPH supported twofold more activity in the microsomal fraction. The data further suggested that the increased activity provided by NADH in the 9000*g* supernatant was due to the presence of endogenous $NADP^+$ and an NADPH-generating system. Nicotinamide was also required, probably because it protects against NADP degradation (24–29).

Data in Table 3 show that the dehydrogenase system was not affected by addition of 4 mmol EDTA or $MgCl_2$ up to 16 μmol. Cyanide, which is known to inhibit other

TABLE 3

Inhibition of Dehydrogenase Activity

Incubation medium[a]	Relative dehydrogenase activity
Complete 9000*g* supernatant	100
+Mg Cl_2 (16 μmol)	100
+EDTA (4 mmol)	100
+NaCN (5 μmol)	106
+SKF 525-A (1 mmol)	18
+SKF 525-A (100 μmol)	63
+O_2 (bubbled gently 1 min)	100
+N_2 (bubbled gently 1 min)	0
+CO (bubbled gently 1 min)	0

[a] "Complete" incubation medium and conditions as described in Methods.

dehydrogenases (30, 31), had little effect on the dehydrogenase system that metabolizes lindane. Though not included in Table 3, glutathione, cysteine, dithiothreitol, and EDTA, which have been used to stabilize or enhance the activity of various dehydrogenases (32–34) had no effect on the dehydrogenation of lindane. Results from Table 3 indicate that SKF 525-A, which by binding to microsomes and soluble cytochrome P-450 greatly inhibits interactions of substrates with cytochrome P-450 (35), was a potent inhibitor of the dehydrogenase activity. Furthermore, dehydrogenase activity was completely inhibited following a brief (1 min) exposure to either CO or N_2. When N_2 was used, lindane was metabolized by a different route to a different metabolite, which was tentatively identified by mass spectroscopy as γ-3,4,5,6-tetrachlorocyclohex-1-ene and the enzyme system appeared to be similar to that reported by Walker (36). Various CO/O_2 ratios were used to determine *K*, the Warburg partition coefficient (37), which is the CO/O_2 ratio required for 50% inhibition of the dehydrogenase system. The ratio was found to be 1.8 (Fig. 6) which is in line with CO/O_2 ratios required for 50% inhibition of various other hepatic microsomal mixed function oxidases (38).

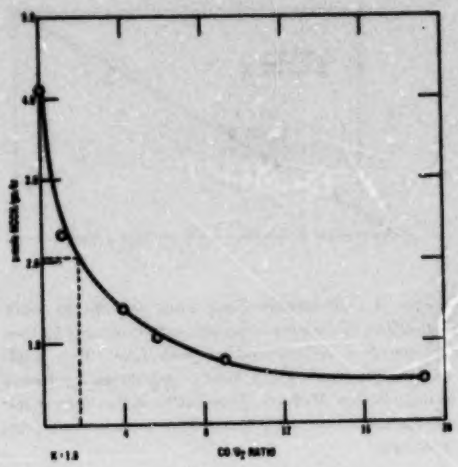

FIG. 6. *Effect of varying the CO/O_2 atmosphere on the* in vitro *metabolism of lindane to hexachlorocyclohexene (μmole/gram liver/hour). The Warburg partition coefficient K = the CO/O_2 ratio required for 50% inhibition of the metabolism of lindane to hexachlorocyclohexene. Incubation conditions as described in Methods. Each value is the mean of two determinations.*

Specificity of the Dehydrogenase System

Both γ-chlordane (1-exo-2-endo-4,5,6,7,-8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene) and α-chlordane (1-exo-2-exo-4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene) are metabolized *in vitro* by dehydrogenation to the intermediate 1,2,-dichlorochlordene (39). Since rat liver preparations incorporating an NADPH-generating system were utilized in this work, it seemed possible that lindane was being metabolized by the same system. As illustrated in Table 4, neither γ- nor α-chlordane appeared to significantly inhibit the dehydrogenation of lindane, and lindane, in turn, had little effect on the dehydrogenation of either chlordane isomer. Previous work in this laboratory has demonstrated that when separate groups of rats are administered one of four configurational isomers of hexachlorocyclohexane (α-, β, γ-, or δ-HCH) only the group receiving the γ-HCH excreted PCCOL, 2,3,4,6-, and 2,3,4,5-tetrachlorophenol (40). Since these

TABLE 4

Specificity of the Dehydrogenase System from Rat Liver

Substrates utilized[a]	Relative dehydrogenase activity	
	Hexachlorocyclohexene	Dichlorochlordene
γ-HCH	100	
α-chlordane		100
α-chlordane + γHCH	87	89
γ-chlordane		100
γ-chlordane + γHCH	83	94
α-HCH + γHCH	84	
β-HCH + γHCH	88	
δ-HCH + γHCH	54	

[a] One hundred micrograms of each substrate was used in the standard incubation mixture described in Methods.

metabolites could result from a pathway involving a stereospecific dehydrogenase, the metabolism of combinations of lindane (γ-HCH) with the α-, β-, and δ-isomers were compared to that of lindane alone for competitive inhibition of the dehydrogenase system. Only the δ-HCH significantly inhibited the *in vitro* dehydrogenation of lindane indicating that the dehydrogenase exhibited some stereospecificity (Table 4).

In Vivo Metabolism of Hexachlorocyclohexene

Rats administered synthetic HCCH, excreted all previously identified lindane metabolites for which adequate analytical procedures have been developed (Table 5). Moreover, the total metabolism of HCCH to chlorophenols and PCCOL took place almost twice as readily as that of lindane. However, while the quantities of 2,3,5-, 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol excreted by rats receiving HCCH were significantly greater than that excreted by lindane treated rats, the lindane treated animals excreted more 2,4,6-trichlorophenol. No significant differences in the excretion of 2,3,4,5-tetrachlorophenol or PCCOL by the two groups were noted.

In Vitro Metabolism of Hexachlorocyclohexene

The *in vitro* incubation of HCCH with liver preparations produced PCCOL and all five of the chlorophenols recovered from the urine of rats treated with either lindane or hexachlorocyclohexene (Table 6). Pretreatment of the rats with DDT significantly induced the metabolism of HCCH to all of these metabolites. The amount of PCCOL formed was at least five times greater than any of the other metabolites formed *in vitro*, whereas *in vivo* the reverse was true. This could indicate that PCCOL was metabolized further *in vivo* or it could be explained by the fact that optimum incubation conditions were not employed for the other enzymes responsible for HCCH metabolism *in vitro*.

DISCUSSION

Currently, it is accepted that dehydrochlorination of lindane to γ-PCCH is the critical initial step in the metabolism of this organochlorine insecticide by mammals (16–18). However, it is difficult to account for the chemical structure of some previously identified lindane metabolites by a metabolic pathway in which dehydrochlorination is the first step. For example, it was necessary to hypothesize an allylic oxidation with a double bond shift to account for the formation of PCCOL from γ-PCCH in the rat (18), and no attempt has been made to account for the metabolism of lindane to pentachlorophenol in rabbits. Furthermore, a previous report from this laboratory indicated that γ-PCCH is metabolized by the rat to only 2,4,5- plus a trace of 2,3,5-trichlorophenol and not to PCCOL or to the other chlorophenols (40). On the other hand, HCCH is metabolized by the rat to PCCOL and all of the chlorophenols produced from lindane (Tables 5

TABLE 5

Comparative in vivo Metabolism of Lindane and HCCH

Compound administered[a]	Average μg metabolite excreted/24 H ± SE[b]					
	246-TCP[c]	235-TCP[d]	245-TCP[e]	2346-TTCP[f]	2345-TTCP[g]	PCCOL[h]
Lindane	51.1 ± 8.27	8.43 ± 0.981	14.7 ± 3.22	27.1 ± 4.06	4.32 ± 2.82	0.25 ± 0.067
HCCH	20.6 ± 4.10[i]	33.6 ± 2.50[i]	50.1 ± 4.24[i]	86.8 ± 11.2[i]	5.39 ± 0.990	0.48 ± 0.120

[a] Two milligrams of the respective compound/0.1 ml peanut oil was administered to each rat.
[b] Excretion values represent the mean of six animals ± SE.
[c] 2,4,6-Trichlorophenol.
[d] 2,3,5-Trichlorophenol.
[e] 2,4,5-Trichlorophenol.
[f] 2,3,4,6-Tetrachlorophenol.
[g] 2,3,4,5-Tetrachlorophenol.
[h] 2,3,4,5,6-Pentachloro-2-cyclohexen-1-ol.
[i] $P < 0.05$.

and 6). A pathway involving the initial dehydrogenation of lindane to HCCH should significantly simplify attempts to account for known lindane metabolites in mammals.

Apparently, this is the first report of a cytochrome P-450 dependent mixed function oxidase which requires molecular oxygen and reduced pyridine nucleotide coenzyme, and catalyzes the dehydrogenation of a pesticide. Although other microsomal dehydrogenases have been reported (41–46), these do not require cytochrome P-450 or reduced pyridine nucleotide coenzyme. Though both systems require molecular oxygen and reduced pyridine nucleotide coenzyme, the mixed-function oxidase responsible for lindane dehydrogenation differs from the fatty acid desaturase described by Oshino (31) in that the dehydrogenation of lindane is cytochrome P-450 dependent and not sensitive to cyanide inhibition, whereas the opposite holds true for the desaturase system.

It is possible that the dehydrogenase system metabolizing lindane is the same

TABLE 6

In Vitro Metabolism of Hexachlorocyclohexene by Rat Liver[a]

Animal pretreatment[b]	Average μg metabolite produced/30 min					
	246-TCP[c]	235-TCP[d]	245-TCP[e]	2346-TTCP[f]	2345-TTCP[g]	PCCOL[h]
Control	2.2 ± 0.12	0.74 ± 0.10	1.3 ± 0.07	4.3 ± 0.40	0.50 ± 0.06	25 ± 3.5
DDT	4.8 ± 0.05[i]	2.1 ± 0.18[i]	6.0 ± 0.23[i]	11 ± 0.36[i]	0.92 ± 0.04[i]	40 ± 2.9[i]

[a] Four hundred micrograms of HCCH was used in the standard incubation mixture described in Methods.
[b] Pretreatment as described in Methods.
[c] 2,4,6-Trichlorophenol.
[d] 2,3,5-Trichlorophenol.
[e] 2,4,5-Trichlorophenol.
[f] 2,3,4,6-Tetrachlorophenol.
[g] 2,3,4,5-Tetrachlorophenol.
[h] 2,3,4,5,6-Pentachloro-2-cyclohexen-1-ol.
[i] $P < 0.05$.

as that which converts α- and γ-chlordane to 1,2-dichlorochlordene (39) or that which dehydrogenates pyrrolizidine alkaloids (47). However, the intracellular distribution and cofactor requirements of the enzyme which dehydrogenates chlordane have not been reported. Furthermore, saturating levels of either α- or γ-chlordane fail to significantly inhibit the dehydrogenation of lindane *in vitro*, and lindane has little effect on the dehydrogenation of either chlordane isomer (Table 4). This suggests that either different dehydrogenases or different binding proteins for the same dehydrogenase may be involved here. Similarities between the microsomal enzyme catalyzing the dehydrogenation of lindane and that reported to convert pyrrolizidine alkaloids to dihydropyrrolizine derivatives include a requirement for molecular oxygen, reduced $NADP^+$, and inhibition by CO and SKF 525-A together with induction by DDT pretreatment (47). The authors of that study indicate that a new type of microsomal reaction need not be hypothesized. They propose a mechanism for the dehydrogenation of pyrrolizidine alkaloids which might involve initial C-hydroxylation or N-demethylation followed by a spontaneous sequence of nonenzymatic reactions. Such a mechanism, however, is not applicable to the metabolism of lindane. Moreover, the fact that the dehydrogenation of lindane apparently does involve a previously unreported type of microsomal reaction leads to speculation that the pyrrolizidine alkaloids and lindane may actually be metabolized by the same enzyme system. The metabolic dehydrogenation of these alkaloids produces toxic pyrrole derivatives and the detoxification of at least two pesticides, lindane and chlordane, may involve the same pathway. This enzyme system should be further characterized to determine its possible implication in the toxicology and metabolism of other xenobiotics.

Hamilton has suggested that the oxenoid mechanism may be involved in the dehydrogenation catalyzed by microsomal enzymes (48). On the basis of analogies with carbene (49) and nitrene (50) reactions, in addition to model reaction studies, Hamilton proposed that the equivalent of a free oxygen atom, with its six outershell electrons, abstracts two hydrogens to give water and the alkene directly (Fig. 7).

The oxenoid reagent $E - X = O$ is generated from O_2 and a group on the enzyme by reduction. Hamilton has speculated that the structure of the oxenoid reagent could be a peracid or a peramide formed from a carboxyl or amide group and the superoxide ion $O_2^{-}\cdot$, or Fe (III)$\cdot O_2^{-}\cdot$. In numerous examples of the oxidative formation of nonactivated double bonds (conjugated at the most to a second double bond), the removal of two hydrogens occurs by a *cis* elimination and is consistent with the oxenoid mechanism (51–62). This list includes double bond formation in steroid rings, steroid side chains, and fatty acids.

It is proposed that the dehydrogenation of lindane by a hepatic, cytochrome P-450 dependent mixed function oxidase represents an important initial step in the metabolic pathway leading to the detoxification of this pesticide by mammals.

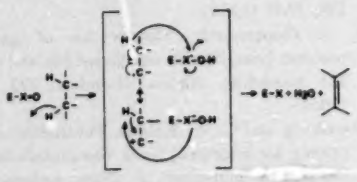

FIG. 7. *Possible mechanism for the dehydrogenation of lindane by microsomal cyt. P-450. E−X=O represents the oxenoid reagent, generated from O_2 and a group on the enzyme, which abstracts two hydrogens from a substrate such as lindane to give water and the alkene directly.*

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